REMARKS

Claims 1-11 are pending in this application. Claims 1-7 have been withdrawn from consideration. Claims 8-11 have been rejected. Claims 1-7, 10, and 11 have been canceled. Claims 8 and 9 have been amended. No new matter has been added by these amendments to the claims. Reconsideration is respectfully requested in light of the claim amendments and the following remarks.

I. Restriction Requirement

The Restriction Requirement placing claim 1 into Group I, claims 2-7 into Group II, and claims 8-11 into Group III has been deemed proper and made Final. Accordingly, Applicants have canceled claims 1-7 without prejudice, reserving the right to file continuing applications on the canceled subject matter.

II. Rejection of Claims Under 35 U.S.C. 112, First Paragraph

Claims 10 and 11 have been rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The Examiner suggests that the claims contain subject matter which was not described in the specification in such a way as to enable one skilled in the relevant art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. The Examiner suggests that the specification fails to teach one of skill how to practice the claimed invention as a method of preventing disease or disorders associated with over accumulation of cholesterol because "prevention" which provides an expectation that the disorder or condition does not occur when challenged, is not taught in the

specification as filed. In an earnest effort to advance the prosecution of this case, Applicants have canceled claims 10 and 11. Accordingly, withdrawal of this rejection is respectfully requested.

III. Rejection of Claims Under 35 U.S.C. 112, Second Paragraph

Claims 8-11 have been rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner suggests that the claims are vague and indefinite because they depend on withdrawn claims. Applicants have amended claims 8 and 9 and canceled claim 10 and 11. Claims 8 and 9 are now independent claims and do not rely on withdrawn claims. Support for the amendments to the claims can be found throughout the specification as filed, in particular at pages 12-17. Withdrawal of this rejection is therefore respectfully requested.

IV. Rejection of Claims Under 35 U.S.C. 102

Claims 8-11 have been rejected under 35 U.S.C. 102(b) as being anticipated by Deboeck et al. (U.S. Patent No. 5,545,628). The Examiner suggests that this reference discloses methods for treating hyperlipidemia or hypercholesterolemia, an over accumulation of cholesterol, by administering fenofibrate to a mammal and although the reference does not teach the compound can be identified by the method of the instant invention, this compound which reduces accumulation of cholesterol must be identifiable via the claimed method. Applicants respectfully

disagree with the Examiner's conclusions regarding this reference.

At the outset, as discussed *supra*, claims 10 and 11 have been canceled making this rejection moot as it pertains to those claims. Additionally, as discussed *supra*, Applicants have amended claims 8 and 9 to recite that the present invention is methods for inhibiting accumulation of cholesterol inside cells which comprise administering cholesterol inhibitors that have been identified using a very specific process that is based on identification of a specific mechanism of action for the cholesterol inhibitor. This mechanism of cholesterol inhibition is as new finding as described in detail in the specification as filed.

U.S. Patent No. 5,545,628 discloses the use of fenofibrate in a specific composition or formulation to treat hypercholesterolemia in patients. This patent is directed to a new formulation for a drug (fenofibrate) that is now approved for use in humans by the US Food and Drug Administration. The Examiner suggests that since this drug is used to treat hypercholesterolemia and hyperlipidemia that it would have been identified as pharmacologically active using the method of the present invention. However, Applicants respectfully point out that the method to be used to identify the cholesterol inhibitors of the present invention is a unique method based on a unique mechanism of action of these cholesterol inhibitors. As a result, compounds useful in the present invention are limited, as now claimed, to compounds that act to inhibit accumulation of cholesterol inside cells by this very specific mechanism (*i.e.*, compound that block the internalization of LDL-derived

cholesterol and/or plasma membrane cholesterol from entering the cell interior thereby causing cholesterol to accumulate in the plasma membrane and promoting cholesterol efflux and stimulating reverse cholesterol transport in various body cells; as taught at page 18, lines 15-21 of the specification as filed). The Examiner fails to recognize that the compound taught in U.S. Patent No. 5,545,628, fenofibrate, acts by a very different mechanism to affect cholesterol homeostasis and prevent hypercholesterolemia and hyperlipidemia. This fenofibrate-specific mechanism is identified in pharmacology textbooks that would be used by one of skill in the art. For example, in one of the most well-known pharmacology textbooks, *Goodman & Gilman's The Pharmacological Basis of Therapeutics* (2001. Hardman and Limbird (eds.), 10th edition, chapter 36, page 993, enclosed herewith) it is taught that fenofibrate although the exact mechanism by which fenofibrate operates in humans is still unclear, the mechanism of action does appear to be related to the interaction of the drug with peroxisome proliferators-activated receptors (PPARs). These receptors regulate gene transcription and fenofibrate acts to reduce triglyceride levels in blood through PPAR-mediated stimulation of fatty acid oxidation. Fenofibrate and other fibrates also increase lipoprotein lipase synthesis and reduce expression of apoC-III, which serves as an inhibitor of processes that enhance clearance of very low density lipoprotein (VLDL) from blood. Nowhere is it taught or suggested that any part of the mechanism of action of fenofibrate is related to blocking the internalization of LDL-derived cholesterol and/or plasma membrane cholesterol from entering the cell interior thereby causing cholesterol to accumulate in the

plasma membrane and promoting cholesterol efflux and stimulating reverse cholesterol transport in various body cells, as taught in the specification as filed. Therefore, one of skill would not expect that fenofibrate would be useful as a compound to inhibit cholesterol accumulation in cells intracellularly nor to be identified as a cholesterol inhibitor as claimed.

MPEP 2131 teaches that in order to anticipate an invention the cited reference must teach each and every limitation of the claims. Clearly, the cited patent which teaches use of fenofibrate in a new formulation fails to teach the limitations of the claims as amended which recite that the cholesterol inhibitor used is identified by a specific method that is related to a mechanism of action that is entirely different from the mechanism of action of fenofibrate. Accordingly, the cited patent cannot anticipate the invention of the claims as amended. Withdrawal of this rejection is respectfully requested.

Claims 8-11 have been rejected under 35 U.S.C. 102(a) and 102(e) as being anticipated by Chang (WO 02/068681) and 102(e) as being anticipated by Chang (US Patent Application 2004/0115613). In both cases, the Examiner suggests that although the references fail to disclose compounds that can be identified by the exact methods claimed, the compounds identified in the references are those which modulate, inhibit and reduce accumulation of cholesterol and as such would be identifiable via the claimed methods. Applicants respectfully traverse these rejections.

Both of the references cited by the Examiner disclose methods for identifying cholesterol inhibitors. However, neither of the patent applications referenced teach or suggest the method as now specified in the claims as amended which relies on the use

of a screening method for cholesterol inhibitors that involves permeabilization of cells and evaluating the intracellular level of cholesterol accumulation in the permeabilized mutant *NPC1* cells exposed to the test agent via binding of labeled C theta complex to cholesterol-rich domains in intracellular organelles. MPEP 2131 states that in order to anticipate an invention the cited reference must teach each and every limitation of the claims. Clearly, these cited references fail to teach or suggest the specific limitations of the claims as amended. Accordingly, the cited references fail to anticipate the invention of the amended claims. Withdrawal of these rejections under 35 U.S.C. 102 are, therefore, respectfully requested.

V. Conclusions

Applicants believe that the foregoing comprises a full and complete response to the Office Action of record. Accordingly, favorable reconsideration and subsequent allowance of the pending claims is earnestly solicited.

Respectfully submitted,

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Fibric Acid Derivatives

History. Thorp and Waring (1962) reported that ethyl chlorophenoxyisobutyrate lowered lipid levels in rats. In 1967, the ester form (*clofibrate*) was approved for use in the United States and was, for a number of years, the most widely prescribed hypolipidemic drug. Its use declined dramatically, however, after the results of the World Health Organization (WHO) trial were published in 1978. This trial found that, despite a 9% reduction in cholesterol levels, clofibrate treatment did not reduce fatal cardiovascular events, although nonfatal infarcts were reduced (Committee of Principal Investigators, 1978). Total mortality was significantly greater in the clofibrate group. The increased mortality was due to multiple causes, including cholelithiasis. Interpretation of these negative results was clouded by failure to analyze the data according to the intention-to-treat principle. A later analysis demonstrated that the apparent increase in noncardiac mortality did not persist in the clofibrate-treated patients after discontinuation of the drug (Heady *et al.*, 1992). Clofibrate use was virtually abandoned after the 1978 WHO trial publication, although it, as well as two other fibrates, *gemfibrozil* and *fenofibrate,* remain available in the United States.

Two subsequent trials, the Helsinki Heart Study and the Veterans Affairs HDL Intervention Trial, have reported favorable effects of gemfibrozil therapy on fatal and nonfatal cardiac events without an increase in morbidity or mortality (Frick *et al.*, 1987; Rubins *et al.*, 1999).

Chemistry. Clofibrate, the prototype of the fibric acid derivatives, is the ethyl ester of *p*-chlorophenoxyisobutyrate. Gemfibrozil is a nonhalogenated phenoxypentanoic acid and thus is distinct from the halogenated fibrates. A number of fibric acid analogs (*e.g., fenofibrate, bezafibrate,* and *ciprofibrate*) have been developed and are used in Europe and elsewhere (*see* Figure 36–5 for structural formulas).

Mechanism of Action. Despite extensive studies in human beings, the mechanisms by which fibrates lower lipoprotein levels, or raise HDL levels, remain unclear (Grundy and Vega, 1987; Illingworth, 1991). Recent studies suggest that many of the effects of these compounds on blood lipids are mediated by their interaction with peroxisome proliferator–activated receptors (PPARs) (Kersten *et al.*, 2000), which regulate gene transcription. Three PPAR isotypes (α, β, and γ) have been identified. Fibrates bind to PPARα, which is expressed primarily in the liver and brown adipose tissue and to a lesser extent in the kidney, heart, and skeletal muscle. Fibrates reduce triglycerides through PPARα-mediated stimulation of fatty acid oxidation, increased LPL synthesis, and reduced expression of apoC-III. An increase in LPL would enhance the clearance of triglyceride-rich lipoproteins. A reduction in hepatic production of apoC-III, which serves as an inhibitor of lipolytic processing and receptor-mediated clearance, would enhance the clearance of VLDL. Fibrate-mediated increases in HDL-C are due to PPARα stimulation of apoA-I and apoA-II expression (Staels and Auwerx, 1998), which increases HDL levels.

LDL levels rise in many patients, especially hypertriglyceridemic patients, treated with gemfibrozil. However, LDL levels are unchanged or fall in others, especially those whose triglyceride levels are not elevated or who are taking a second-generation agent, such as fenofibrate, bezafibrate, or ciprofibrate. The fall of LDL levels may be due in part to changes in the cholesterol and triglyceride contents of LDL that are mediated by cholesteryl ester transfer protein activity; such changes can alter the affinity of LDL for the LDL receptor (Eisenberg *et al.*, 1984). There also is evidence that a PPARα-mediated increase in hepatic SREBP-1 production enhances hepatic expression of LDL receptors (Kersten *et al.*, 2000). Lastly, gemfibrozil reduces the plasma concentration of small, dense, more easily oxidized LDL particles (Yuan *et al.*, 1994).

Most of the fibric acid agents have potential antiatherothrombotic effects, including inhibition of coagulation and enhancement of fibrinolysis. These salutary effects also could alter cardiovascular outcomes by mechanisms unrelated to any hypolipidemic activity (Watts and Dimmitt, 1999).

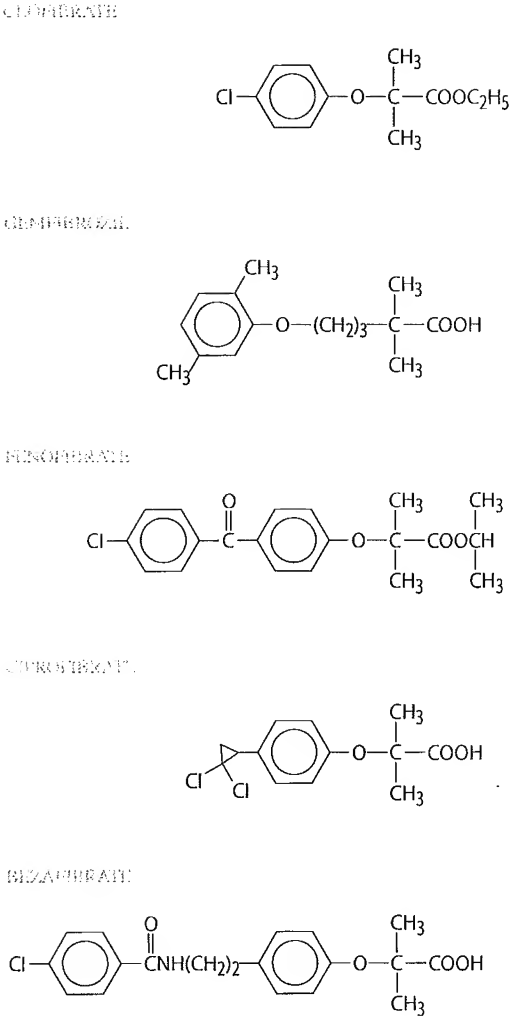

Figure 36–5. Structures of the fibric acids.

Effects on Lipoprotein Levels. The effects of the fibric acid agents on lipoprotein levels differ widely depending on the

starting lipoprotein profile, the presence or absence of a genetic hyperlipoproteinemia, the associated environmental influences, and the drug used.

Patients with type III hyperlipoproteinemia (dysbetalipoproteinemia) are among the most sensitive responders to fibrates (Mahley and Rall, 2001). Elevated triglyceride and cholesterol levels are dramatically lowered, and tuberoeruptive and palmar xanthomas may regress completely. Angina and intermittent claudication also improve (Kuo *et al.,* 1988).

In patients with mild hypertriglyceridemia (*e.g.,* triglycerides <400 mg/dl), fibrate treatment decreases triglyceride levels by up to 50% and increases HDL-C concentrations about 15%; LDL-C levels may be unchanged or increase. The second-generation agents, such as fenofibrate, bezafibrate, and ciprofibrate, lower VLDL levels to a degree similar to that produced by gemfibrozil, but they also are more likely to decrease LDL levels by 15% to 20%. In patients with more marked hypertriglyceridemia (*e.g.,* 400 to 1000 mg/dl), a similar fall in triglycerides occurs, but LDL increases of 10% to 30% are seen frequently. Normotriglyceridemic patients with heterozygous familial hypercholesterolemia usually experience little change in LDL levels with gemfibrozil; with the other fibric acid agents, reductions as great as 20% may occur in some patients.

Fibrates usually are the drugs of choice for treating severe hypertriglyceridemia and the chylomicronemia syndrome. While the primary therapy is to remove alcohol and as much fat from the diet as possible, fibrates help both by increasing triglyceride clearance and by decreasing hepatic triglyceride synthesis. In patients with chylomicronemia syndrome, fibrate maintenance therapy and a low-fat diet keep triglyceride levels well below 1000 mg/dl and thus prevent episodes of pancreatitis.

Gemfibrozil was used in the Helsinki Heart Study, a primary prevention trial of 4081 hyperlipidemic men who received either placebo or gemfibrozil for 5 years (Frick *et al.,* 1987). Gemfibrozil reduced total cholesterol by 10% and LDL-C by 11%, raised HDL-C levels by 11%, and decreased triglycerides by 35%. Overall, there was a 34% decrease in fatal and nonfatal cardiovascular events without any effect on total mortality. No increased incidence of gallstones or cancers was observed. Subgroup analysis suggested that the greatest benefit occurred in the subjects with the highest levels of VLDL or combined VLDL and LDL and in those with the lowest HDL-C levels (<35 mg/dl). It also is possible that gemfibrozil affected the outcome by influencing platelet function, coagulation factor synthesis, or LDL size. In a recent secondary prevention trial, gemfibrozil reduced fatal and nonfatal CHD events by 22% despite a lack of effect on LDL-C levels. HDL-C levels increased by 6%, which may have contributed to the favorable outcome (Rubins *et al.,* 1999).

Absorption, Fate, and Excretion. All of the fibrate drugs are absorbed rapidly and efficiently (>90%) when given with a meal but less efficiently when taken on an empty stomach. The ester bond is hydrolyzed rapidly, and peak plasma concentrations are attained within 1 to 4 hours. More than 95% of these drugs in plasma are bound to protein, nearly exclusively to albumin. The half-lives of fibrates differ significantly (Miller and Spence, 1998), ranging from 1.1 hours (gemfibrozil) to 20 hours (fenofibrate). The drugs are widely distributed throughout the body, and concentrations in liver, kidney, and intestine exceed the plasma level. Gemfibrozil is transferred across the placenta. The fibrate drugs are excreted predominantly as glucuronide conjugates; 60% to 90% of an oral dose is excreted in the urine, with smaller amounts appearing in the feces. Excretion of these drugs is impaired in renal failure, though excretion of gemfibrozil was reported to be less severely compromised in renal insufficiency than was excretion of other fibrates (Evans *et al.,* 1987). Nevertheless, the use of fibrates is contraindicated in patients with renal failure.

Adverse Effects and Drug Interactions. Fibric acid compounds usually are well tolerated (Miller and Spence, 1998). Side effects may occur in 5% to 10% of patients but most often are not sufficient to cause discontinuation of the drug. Gastrointestinal side effects occur in up to 5% of patients. Other side effects are reported infrequently and include rash, urticaria, hair loss, myalgias, fatigue, headache, impotence, and anemia. Minor increases in liver transaminases and decreases in alkaline phosphatase have been reported. Clofibrate, bezafibrate, and fenofibrate have been reported to potentiate the action of oral anticoagulants, in part by displacing them from their binding sites on albumin. Careful monitoring of the prothrombin time and reduction in dosage of the anticoagulant may be appropriate when treatment with a fibrate is begun.

A myositis flu-like syndrome occasionally occurs in subjects taking clofibrate, gemfibrozil, and fenofibrate, and may occur in up to 5% of patients treated with a combination of an HMG-CoA reductase inhibitor and gemfibrozil, if higher doses of the reductase inhibitor are used. Patients on this combination should be instructed to be aware of the potential symptoms and should be followed at 3-month intervals with careful history and determination of CK values until a stable pattern is established. Use of fibrates with cerivastatin should be avoided because a number of cases of rhabdomyolysis have resulted from combined gemfibrozil-cerivastatin therapy (Guyton *et al.,* 1999; Alexandridis *et al.,* 2000).

All of the fibrates increase the lithogenicity of bile. In the Coronary Drug Project and the WHO trial, clofibrate use was associated with increased risk of gallstone formation. However, no significant increase was seen in the Helsinki Heart Study with the use of gemfibrozil or in the VA HIT. Placebo-controlled clinical trial data for fenofibrate are not available.

Renal failure is a relative contraindication to the use of fibric acid agents, as is hepatic dysfunction. Combined statin-fibrate therapy should be avoided in patients with compromised renal function. Gemfibrozil should be used with caution and at a reduced dosage to treat the hyperlipidemia of renal failure. Fibrates should not be used by children or pregnant women.

Therapeutic Uses. Clofibrate (ATROMID-S) is available for oral administration. The usual dose is 2 g per day in divided doses. This compound is little used, but it still may be useful in patients who do not tolerate gemfibrozil or fenofibrate. Gemfibrozil (LOPID) is usually administered as a 600-mg dose taken twice a day 30 minutes

before the morning and evening meals. Fenofibrate (TRICOR) is available as single-dose capsules of 67, 134, and 200 mg.

Fibrates are the drugs of choice for treating hyperlipidemic subjects with type III hyperlipoproteinemia as well as subjects with severe hypertriglyceridemia (triglycerides >1000 mg/dl), who are at risk for pancreatitis. Based on the VA HIT results, fibrates appear to have an important role in subjects with familial combined hyperlipidemia, who predominantly have elevated VLDL levels and low HDL-C levels. When fibrates are used in such patients, the LDL levels need to be monitored; if LDL levels rise, the addition of a low dose of a statin may be needed. Alternatively, many experts now treat such patients first with a statin, and only subsequently add a 600-mg dose of gemfibrozil once or twice a day to further lower VLDL levels. If this combination is used, there should be careful monitoring for myositis.

PROSPECTUS

New Lipid-Lowering Agents

Statins. More potent statins capable of lowering LDL-C by >65% are being developed. Some of these agents also may have greater efficacy in reducing triglycerides and raising HDL-C. A new statin, ZD4522, in developmental clinical trials has been reported to reduce LDL-C by 65% (Olsson *et al.,* 2000).

MTP Inhibitor. MTP transfers triglycerides and other nonpolar lipids to the apolipoproteins of nascent lipoproteins as they form in the intestine and liver and is required for the synthesis and secretion of chylomicrons and VLDL. For example, an MTP inhibitor targeted to the liver would decrease VLDL production, thereby decreasing plasma triglyceride levels and ultimately reducing LDL production from VLDL. One such MTP inhibitor, BMS-201038, is in clinical trials (Wetterau *et al.,* 1998).

Dietary and Biliary Cholesterol Absorption Inhibitor. *Ezetimibe* is an azetidione-based cholesterol absorption inhibitor that blocks the intestinal absorption of cholesterol, resulting in lowered plasma total cholesterol and LDL-C levels (van Heek *et al.,* 2000). The drug undergoes glucuronidation in the intestine, and the absorbed glucuronide, an active metabolite, is excreted into the bile by the liver. Due to its enterohepatic circulation, the half-life of the active metabolite is 22 hours in human beings, which indicates that once daily dosing is sufficient (Zhu *et al.,* 2000). The maximum effective dose is 10 mg daily, which provides a 19% reduction in LDL-C (Lipka *et al.,* 2000). A 19% reduction of LDL-C by ezetimibe is equivalent to three doublings of a statin from its baseline dose, since each doubling of a statin dose after the starting dose provides an additional 6% decrease in LDL-C (Pedersen and Tobert, 1996). In human beings, the combination of simvastatin (20 mg; the starting dose of simvastatin) plus ezetimibe (10 mg) provides a 52% reduction in LDL-C (Kosoglou *et al.,* 2000). This 50% reduction achieved with low-dose simvastatin plus ezetimibe (10 mg) is equivalent to that of 80 mg of simvastatin alone. Since there is virtually no myopathy when statins are used at their starting doses, the combination of ezetimibe plus low-dose statin promises an additional margin of safety. Early studies suggest that there are no dangerous interactions between ezetimibe and statins, but further investigations are required to adequately document this initial impression.

ACAT Inhibitors. To date, ACAT inhibitors have failed to reach the marketplace. However, the recognition that there are at least two forms of this enzyme with specific tissue sites of expression suggests that it may be possible to develop an inhibitor that specifically inhibits the assimilation of dietary cholesterol.

ACAT-1 is expressed in several tissues, including macrophages (Chang *et al.,* 1993). *Avasimibe,* an inhibitor of this enzyme, appears to reduce macrophage and cholesteryl ester contents of lesions in cholesterol-fed rabbits and could affect atherosclerotic lesion development, an effect that could stabilize lesions (Bocan *et al.,* 2000). Avasimibe reduced plasma triglycerides and LDL-C levels by up to 50% in miniature swine; however, hepatic triglyceride content increased two- to sevenfold in a dose-dependent manner (Burnett *et al.,* 1999). Interestingly, ACAT-1 knockout mice did not have reduced susceptibility to developing atherosclerosis (Accad *et al.,* 2000).

ACAT-2 is expressed in the liver and intestine and appears to play a role in cholesteryl ester formation for VLDL and chylomicron production (Cases *et al.,* 1998). An inhibitor of this form of ACAT could reduce plasma lipids.

For further discussion of hyperlipoproteinemias and other disorders of lipid metabolism, *see* Chapters 75, 242, and 341 in *Harrison's Principles of Internal Medicine,* 14th ed., McGraw-Hill, New York, 1998.